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(54) Antibodies and antibody-containing compositions for inhibiting dental caries

(57) Antibodies for inhibiting human dental caries induced by *Streptococcus mutans* may be prepared by immunizing a mammal with at least one antigen selected from two antigens, a first of which may be isolated from the pili-like structures on the cell surface of strains of *S. mutans* of serotypes *c*, *e*, *f* and *g* and a second of which may be isolated from the pili-like structures on the cell surface of strains of *S. mutans* of serotype *d*, and recovering the resultant antibodies from said mammal.

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SPECIFICATION

Antibodies and antibody-containing compositions for inhibiting dental caries induced by Streptococcus mutans

The present invention relates to antibodies and antibody-containing compositions for inhibiting human dental caries induced by *Streptococcus mutans*.

Dental caries is a disease of humans and animals, which is primarily induced on the surface of a toothed by the action of cariogenic oral bacilli and progressively breaks down its structure. Among the various cariogenic oral bacilli, *Streptococcus mutans* is believed to be the most important. It has previously been reported, for example, in Bergy's Manual of Determinative Bacteriology, page 502 (1974) that a relationship exists between dental caries and *S. mutans*, the suggestion being made that *S. mutans* is a similar organism to *S. salivarious*. Although *S. mutans* has not yet been extensively studied and compared with *S. salivarious*, it has been established that *S. mutans* may be clearly distinguished from *S. salivarious* by the fact that *S. salivarious* produces fructans from sucrose whereas *S. mutans* produces both water-soluble and insoluble dextran-like polysaccharides (hereinafter referred to as DPS) from this disaccharide. As is well known, insoluble DPS produced by *S. mutans* adhere to the surface of teeth to form dental plaque. The major proportion of *S. mutans* in the oral flora resides in the plaque and produces lactic acid, which is believed to break down the surface structure of teeth. Other cariogenic oral bacilli also produce lactic acid. However, the lactic acid produced by *S. mutans* is not released from the plaque, but directly accumulates on the surface of teeth.

Strains of *S. mutans* are classified according to the classification proposed by Bratthall et al into 7 serological groups (serotypes), designated *a* to *g*, on the basis of the immunological specificities of their cell wall antigens [Bratthall, Odont. rev. 20:141 (1970) and ibid., 20:23 (1970) and Perch et al, Acta. Path. Microbiol. Scand. Section B, 82:357 (1974)]. A further classification scheme for strains of *S. mutans* has been proposed by Makoto Sato. According to this classification scheme, strains of *S. mutans* are defined as Human I (HI), Human II (HII) or rat (R) type. The following has been reported by Sato:—

(a) the major proportion (96.3%) of *S. mutans* isolated from human hosts is Human I type, and the rest being Human II type;

(b) all strains of *S. mutans* of rat origin are Rat type;

(c) no *S. mutans* can be isolated from mice and guinea pigs:

(d) all strains of *S. mutans* isolated from monkeys and hamsters are Human I type;

(e) Human I, Human II and Rat types correspond respectively to *c*, *e* and *f*, *d* and *g*, and *a* and *b* serotypes of Bratthall's classification [J. of Dental Heath, Vol. 28, No. 2, pages 100-123 (1978) in the Japanese version].

Possible approaches for inhibiting human dental caries induced by *S. mutans* by immunological means have previously been put forward. British Patent No. 1,375,866, for example, discloses a dental vaccine which comprises as antigen at least a part of the cell of a cariogenic strain of *S. mutans*, the charact ristics of which are the same as the characteristics of *Streptococcus mutans* NCTC 10449, a well-recognized representative cariogenic strain present in the oral cavity of humans. However, to our knowledge, it has not yet been reported that such a vaccine has been used to inhibit human dental caries induced by *S. mutans* with good results. Moreover, it is also known that administration of whole cell antigen originating from *S. mutans* to animals is liable to cause various undesirable side effects such as, for example, cross-reaction with the heart muscle antigen, allergic reaction and the like.

British Patent No. 1,505,513 discloses the production of an antibody preparation, which will inhibit dental caries induced by *S. mutans* in rats, by immunizing a cow with heat-inactivated *S. mutans* cells of particular strains to produce corresponding antibodies in the body of the cow and recovering the resultant antibodies in the cow's milk. It is suggested that such antibodies may be administered to humans for inhibition of dental caries by a wide-variety of methods without the occurrence of the above-mentioned undesirable side-effects. In practice, the antibody-containing compositions disclosed in this British Patent No. 1,505,513 are not suitable for inhibiting human dental caries induced by *S. mutans*, since th y lack a high-level of antibodies to human type strains.

The process for preparation of antibodies disclosed in British patent No. 1,505,513 is given in more detail below.

Cultures of *S. mutans* AHT, BHT, 10449 and 6715 of the serological groups (serotypes) *a*, *b*, *c* and *d* respectively were grown in dialyzed tryptose medium and the cells were separated from the culture broth. The cells were washed 5 times with 0.1M phosphate-buffered saline solution and suspended in a similar buffer solution. The cells were inactivated by heating at 60°C for 30 minutes and resuspended to a final concentration of *S. mutans* AHT, BHT, 10449 and 6715 of 5×10^8 cells/ml. The preparation was used to immunize cows. Milk from cows containing antibodies in response to such immunization was collected and dried.

The levels of antibodies in the dried milk capable of binding to *S. mutans* AHT, BHT, 10449 and 6715 were determined as follows:—

50mg of dried milk was dissolved in 1ml of distilled water and 4 sets of serial 2-fold dilutions were made in saline. To each set of dilution tubes was added either formalin-killed *S. mutans* AHT, BHT, 10449 or 6715 at a concentration of 2×10^8 cells/ml. By assaying the endpoint agglutination titres of the samples, the following antibody titres were obtained:--

Antigen employed	*Serotypes*	*Antibody titre*
AHT	a	64
BHT	b	1024
10449	c	<2
6715	d	256

The following compositions were prepared using the dried milk:—

(a) a mouth wash containing the dried milk diluted

with water (x 1-5);

(b) a sugarless confection such as gum, candy or ic cr am containing more than 25% of th dried milk; and

(c) standard cleansing products, powders or tooth paste containing more than 25% of the dried milk.

The dried milk was tested with young rats fed a caries-promoting diet and orally-infected with *S. mutans* AHT, BHT and 6715. Inhibition of dental caries was noted. *S. mutans* 10449 was not included in this test since no antibodies to this strain were previously detected in the dried milk.

The antibody-containing compositions disclosed in British Patent No. 1,505,513 are clearly capable of inhibiting *S. mutans* 6715, which belongs to the group of II type strains found in the oral cavity of humans. However, since the compositions lack antibodies capable of inhibiting *S. mutans* NCTC 10449 (Serotype *c*; Human I type), which, as previously stated, is a well-recognized cariogenic strain in the oral cavity of humans, or any other human I type strain, they cannot be considered suitable as anti-dental caries compositions for human use.

It is known that oral streptococci such as *S. mutans* adhere to the surfaces of teeth and mucous membranes by means of the pili-like structures (fimbriae) on the surface of the cell wall [Gibbons et al, Ann. Rev. Microbiol., 29:19-44 (1975)]. The present invention is based upon the discovery that antigens which we have isolated from the pili-like structures of Human type strains of *S. mutans* (hereinafter referred to as PLS Antigens) may be used to prepare antibodies capable of inhibiting Human type *S. mutans*. Two PLS antigens have been distinguished by a significant difference of serological specificity, hereinafter referred to as PLS Antigen I and PLS Antigen II. PLS Antigen II may be isolated from strains of sereotype *d*, while PLS Antigen I may be isolated from strains of any of the serotypes *c*, *e*, *f* and *g*.

According to one feature of the present invention, there is provided a process for the preparation of antibodies for inhibiting human dental caries induced by *Streptococcus mutans*, which comprises immunizing a mammal with at least one antigen selected from two antigens, a first of which may be isolated from the pili-like structures on the cell surface of strains of *S. mutans* of serotypes *c*, *e*, *f* and *g* and a second of which may be isolated from the pili-like structures on the cell surface of strains of *S. mutans* of serotype *d*, and recovering the resultant antibodies from said mammal.

The present invention further provides compositions for inhibiting human dental caries induced by *S. mutans*, which comprise an effective amount of antibodies raised against at least one PLS antigen, in association with a pharmaceutically acceptable carrier or excipient.

In our experiments, PLS antigen isolated from *Streptococcus mutans* NCTC 10449, which it is known may be found in the oral cavity of humans (serotype *c*; human I type) was subjected to quantitative determination of p:otein (by Hartree's method, using bovine serum albumin as reference) and carbohydrates (by the phenol/sulphuric acid method, using D-glucose as reference), and its molecular weight was determined by gel filtration using Sephadex G-100 (Pharmacia Fine Chemicals AB., Sweden) with referenc to phosphorylase *b*, bovine serum albumin, egg albumin, chymotrypsinogen and the like having known molecular weights. Similar studies were also performed using certain strains of *S. mutans* which are well recognized reference strains such as *S. mutans* Ingbritt (serotype *c*), OMZ176 (serotype *d*), P4 (serotype *e*), OMZ175 (serotype *f*) and K1R (serotype *g*). The results are as follows:—

(1) A PLS antigen is an acidic glycoprotein containing about 15-25% protein (for example, about 20%) and about 75-85% carbohydrates (for example, about 80%) and having a molecular weight of about $6\text{-}9 \times 10^4$ (for example, about 75,000).

(2) Polyacrylamide disc electrophoresis of PLS antigens results in a characteristic broad band towards the cathode.

(3) If sucrose density-gradient ultracentrifugation is carried out with a PLS antigen, the antigen is found in the fractions having a sucrose density of about 10-13% and a specific gravity of about 1.3-1.4.

(4) If gel filtration of a crude PLS antigen solution is carried out using Sepharcyl S-300 or Sephadex G-100 (Pharmacia Fine Chemicals AB, Sweden), two peaks are eluted (monitored by absorbance at 280 nm). The PLS antigen is recovered from the first peak.

(5) A pI value for a PLS antigen of not more than 3.5 is obtained by the isoelectric focussing method according to Versterberg et al using 1% of the carrier ampholyte (pH 3.5-10.0; commercial product of LKB Produkter AB., Sweden).

There is no significant difference between the fractionation patterns of PLS antigens originating from all Human type strains of *S. mutans*.

Although it is possible, if desired, to use either antibodies raised against PLS antigen I or PLS antigen II for inhibiting Human type I and II strains, it is preferred to use antibodies raised against each of these antigens in combination. Combined use does no harm to the preservability and effect of PLS antigen I and PLS antigen II antibodies.

Antibodies obtained by the process of the present invention, when administered orally to humans, are capable of inhibiting the adherence (infection) of Human type strains of *S. mutans* to the surface of teeth. Inhibition of adherence of strains of *S. mutans* to teeth results in coagulation of the cells of the wild strains of *S. mutans*, for example, in the saliva. This leads to death within a relatively short period of time. It is possible to remove coagulated cells of *S. mutans* from the oral cavity without difficulty, for example, by the use of usual dentifrices, gargles, cleansing ag nts and the like.

It has been found that oral administration of an effective amount of antibodies raised against at least one PLS antigen continuously, for example, for several weeks, results in the complete disappearance of wild strains of *S. mutans* from the oral cavity.

The process for the preparation of antibodies may be effected in the following manner.

For the purpose of the present invention, both wild strains and mutant strains of Human type *S. mutans* may be used. it is advantageous to use a strain having a high adhering ability, and the desired strain may

readily be selected, for example, with reference to a high adhering ability to tube wall *in vitro*. In the examples and experiments described hereinafter, two mutant strains of *S. mutans*, viz. *Streptococcus mutans* Mutant Strain K-Dp (FERM-BP No. 317) and *S. mutans* KH2 (FERM-P No. 366) are used. The characteristics of these mutant strains are substantially the same as those of the corresponding wild strains except they have higher adhering ability. Cultures of these mutant strain were deposited with Bikoken (The Fermentation Research Institute of Industrial Science and Technology) on 5th March 1982 and 23rd July 1983 respectively.

Culturing may be effected in conventional manner, preferably under anaerobic conditions, although aerobic culturing may be carried out if desired. Both synthetic and organic media may be used, but liquid media are preferable for mass propagation. Thus, culturing may usually be effected at a temperature of from 23 to 39°C (for example, about 37°C) and at a pH of from 5.6 to 9.0 (for example, about 7) for 24-72 hours. After completion of culturing, the cells are separated from the culture broth, for example, by centrifugation (8000 r.p.m./5-20 min).

The separated cells are suspended in a suitable solution such as, for example, 0.1-1M acetic acid or 0.1-1M phosphate-buffered 0.1-1M sodium chloride solution (pH 6-8), to which a non-ionic surfactant, for example, Triton X-100 (0.01%-0.001%; commercial product of Rohm & Haas Co., U.S.A.) may, if desired, be added. The cell suspension is then treated with ultrasonic waves (for example, 10-20 KHz/5-20 min) to extract the desired PLS antigen. Electron microscopic observation has indicated that by the action of hypertonic solution, it is possible to extract the PLS antigen alone.

Instead of treatment with ultrasonic waves, it is possible to add ammonium sulfate to the cell suspension at a saturation of about 20-70% (for example, 60%), followed by agitation to dissolve the ammonium sulfate. The mixture is allowed to stand at a low temperature (for example, 4°C) for 24-48 hours to form a precipitate containing the desired antigen. After removal of the supernatant, the precipitated fraction is collected and densely suspended in a similar buffer solution (pH 6-8) and dialyzed against a similar buffer solution at a low temperature (for example, 4°C) to remove ammonium sulfate and dialyseable impurities. The residual solution is collected and centrifuged (for example, 8000 r.p.m./20 min) to obtain an extracted solution containing the PLS antigen.

The resultant extracted solution containing the desired PLS antigen may be fractionated and purified in conventional manner, for example, by the sole or combined use of column chromatography, the isoelectric focussing precipitation method, fractional precipitation using cold solvent such as ethanol, the salting-out method using ammonium sulfate and the like. Especially good results may be obtained, for example, by the density gradient centrifugation method.

In order to avoid denaturation of the PLS antigen, the extraction, fractionation and purification may with advantage be effected at a low temperature, for example, below 10°C.

The purified PLS antigen solution may, if desired, be treated with a suitable inactivating agent such as, for example, formalin (0.2-0.02%), followed by dialysis to remove the inactivating agent in conventional manner.

The purified PLS antigen solution is then diluted, for example, with 0.5-1M phosphate-buffered 0.5-1M sodium chloride solution (pH 6-8) to a protein N concentration of 10-50 µg/ml, to which aluminium hydroxide is added at a final concentration of aluminium of about 100-500 µg/ml to absorb the antigen. An antiseptic agent such as, for example, thimerosal (0.05-0.1% w/v) may be added to the antigen solution. In this manner, a PLS antigen solution suitable for immunization of a mammal is obtained.

Instead of aluminium hydroxide, an equal amount of Freund's complete adjuvant may be added to the antigen solution.

The immunization may be effected in conventional manner, for example, by the use of smaller mammals such as mice, rats and guinea pigs or larger mammals such as rabbits, goats, sheep, horses and cattle. The dose of the PLS antigens may vary, depending upon various factors such as, for example, the type of the mammal. However, it is usually possible to administer the antigen solution to smaller animals at a dose of 10-500 µg and to larger mammals at a dose of 100-2000 µg (once daily, calculated as protein N) by injection, for example, under the skin on the back of the mammal. The immunization may be effected, for example, 2-5 times with an interval of 2-5 weeks. If desired, the immunization may be effected by oral administration, for example, at a 5-10 fold dose and the immunization may be continued, for example, for 3-12 days. For example, in the case of a rabbit, a PLS antigen solution containing 100-200 µg of protein N is mixed, for example, with an equal amount of Freund's complete adjuvant and injected under the skin on the back of the animal once daily for 2-5 times with an interval of 2-5 weeks.

10-14 days after final immunization, for example, blood is collected from the mammal in conventional manner, for example, by cardiac puncture and is used for the preparation of a plasma which may, if desired, be further purified to obtain an antiserum. The resultant plasma or antiserum may be preserved at low temperature for an extended period of time and may be administered to humans without further processing.

If desired, it is possible to immunize a mammal with PLS Antigens I and II in combination, although it is preferred to immunise a mammal either with PLS Antigen I or II alone. Antigen I may be derived from ore than one strain of *S. mutans*, the strains having the same serotype or different serotypes, selected from *c, e, f* and *g*.

The compositions of the present invention for inhibiting human dental caries contain as active ingredient PLS antigen I antibodies or PLS antigen II antibodies or a combination of such antibodies. Th carriers or excipients which may be used in compositions of the present invention may be solid, semi-solid or liquid and the compositions will generally be solid, semi-solid or liquid compositions suitable for oral administration. Thus, the compositions may take the

forms of powders, capsules, granules, tablets, drops, syrups, suspensions, emulsions and the like. Examples of suitable solid carriers include lactose, potato- or soluble starch, magnesium stearate, clay and kieselguhr. Suitable liquid carriers are exemplified by water, saline solution, glycerol, almond oil, drink-containing lactic acid-producing bacilli and juice. The compositions may further contain, if desired, bonding agents, stabilizers, emulsifiers, dispersants, antiseptic agents, preservatives, essence and various other additives conventionally used in the art. Preferred examples of the present compositions include dentifrices, gargles, candies, chewing gums, icecreams and the like.

In order to administer a given amount of antibodies of the present invention to humans simply and effectively, the compositions of the present invention may be formulated, for example, in the forms of tablets, capsules, ampoules, baccals, troches and the like. The amount of antibodies contained in such a dosage unit form may vary, depending upon the types of the dosage unit form and the purpose of administration. In one embodiment, such a dosage unit form contains antibodies of the present invention in such an amount that it is enough to administer 1-10 units as hereinafter defined, of the antibodies once daily. Various tests using humans and hamsters have revealed that strains of *S. mutans* may be removed from the oral cavity by, for example, continuous oral administration for several weeks of antibodies raised against at least one PLS antigen at a daily dose of 1-4 units (as hereinafter defined).

Over an extended period of time, for example, 6-12 months, antibodies of the present invention were continuously administered to the oral cavity of hamsters in large amounts. After this, all animals were pathogenically examined and found to be normal.

In this specification, the unit of the antibody titre is expressed by the precipitation value obtained by the double diffusion method with reference to Goldman et al [Isolation and Characterization of Glial Filaments from Human Brain, J. Cell. Biol., 78:426 (1978)].

In the following non-limiting examples and experiments which illustrate the invention, the culturing was effected at 37°C under anaerobic conditions, using commercially available media at their specified pHs, unless otherwise specified.

Example 1

Preparation of *Streptococcus mutans* Mutant Strain K-Dp (FERM-BP No. 317):

Fresh wild strains of *S. mutans* (serotype *c*) were isolated from the oral cavity of a human and cultured for 24 hours by using Dott Heuwitt Broth (20 ml; commercial of Baltimore Laboratories, Inc., U.S.A., hereinafter referred to as BBL.). After completion of culturing, the cells wer s parated fr m the culture broth by centrifugation (8000 r.p.m./20 min.) and then washed 3 times with 100 ml of 0.75M phosphate-buffered 1M sodium chloride solution (pH 6.8) by centrifugation (8000 r.p.m./20 min). The cells were suspended in a similar buffer solution (20 ml) containing 20% nitrogen mustard at a concentration of about 10^8 living cells per ml and wer kept at 37°C until more than 90% of the cells were killed (for about 60-90 min.). The cells which survived wer c llect d and cultured in a similar manner to that described above by using Dott Heuwitt Broth. After completion of culturing, one platinum loop of the culture broth was transferred to TYC agar plate medium [Stoppelaar et al, Archs. Oral Biol., 12:1190-1201 (1976)] for culturing for 24 hours. The culture broth was allowed to stand at ambient temperature for 24 hours in order to select colonies capable of producing a large amount of insoluble DPS. If desired, the above-mentioned procedure may be repeated until a desired strain having a particularly high capacity for producing insoluble DPS is obtained. The resultant strain was designated as *Streptococcus mutans* Mutant Strain K-DP. This mutant strain was orally administered to hamsters over an extended period of time or subcultured by using various known media to confirm that this strain exhibits a very high adhering ability and its characteristics are genetically stable.

Example 2

Preparation of *Streptococcus mutans* Strain KH2 (FERM-BP No. 366):

Fresh wild strains of *S. mutans* (serotype *d*) were isolated from the oral cavity of a human and treated in a similar manner to that described in Example 1 to prepare a mutant strain having a very high adhering ability and genetically stable characteristics. The resultant mutant strain was designated as *Streptococcus mutans* Strain KH2.

Example 3

Preparation of Antibodies using PLS antigen I isolated from *S. mutans* Mutant Strain K-Dp

Streptococcus mutans Mutant Strain K-Dp (FERM-BP No. 317) was cultured by using a seed medium (500 ml) containing polypeptone (1.7%; Wako, Japan), polypeptone S (0.3%; Wako, Japan), yeast extract (0.5%; Difco., U.S.A.), potassium phosphate, dibasic (0.25%) sodium chloride (0.25%) and glucose (0.25%) and having a pH of 7.0-7.8. After completion of culturing, the culture was transferred to a main medium (15000 ml) having the same composition as the seed medium for culturing for 24 hours under the same conditions. After completion of culturing, ammonium sulfate was added to the culture broth at a saturation of 33% and dissolved by agitation. The mixture was allowed to stand at ambient temperature for 24 hours. The supernatant was removed from the mixture. The precipitated fraction was recovered by centrifugation (8000 r.p.m./30 min.) and suspended in 1M phosphate-buffered 0.1M sodium chloride solution (750 ml; pH 8.0). The cell suspension was allowed to stand at 4°C for 72 hours, while stirring gently with 1-5 rotations/min. The suspension was then centrifuged (8000 r.p.m./30 min.) to remove the cells, and ammonium sulfate was added to the supernatant at a saturation of 60%. Ammonium sulfate was dissolved by agitation and the mixture was allowed to stand at 4°C for 48 hours to form a precipitate containing the desired antigen. The supernatant was removed from the mixture and the precipitated fraction was recovered by centrifugation (8000 r.p.m./30 min.). The recovered material was suspended in a similar phosphate-buffered sodium chloride solution to that described above (100 ml) and the resulting suspension was put into a cellophan tube for dialysis at 4°C for more than 48 hours against a similar buffer solution

(more than 5000 ml) to remove ammonium sulfate and dialyseable impurities. The residual solution was centrifuged (8000 r.p.m./30 min.) and the resultant supernatant was collected.

The supernatant (300 ml) was diluted with a similar phosphate-buffered sodium chloride solution at a protein N concentration of 100 µg/ml. The diluted solution (200 ml) was subjected to sucrose density-gradient ultra-centrifugation (sucrose density 5-30%; 3500 r.p.m./18 hours) using a 65P Ultracentrifuge with a zonal rotor 235T (commercially available from Hitachi Limited, Tokyo). The desired antigen was found in the fractions having a sucrose density of about 10-13% and a specific gravity of about 1.31-1.35, the amount of protein N of the desired PLS antigen being about 33-37 µg/ml. The resultant extracted solution containing the desired antigen was put into a cellophane tube and concentrated to reduce the amount to 1/10 by the use of polyvinylpyrrolidone. The concentrated solution was then diluted with 0.75M phosphate-buffered 1M sodium chloride solution (pH 6.2-6.5) to a concentration of protein N of 50-100 µg/ml. To the diluted solution was added an equal amount of Freund's complete adjuvant to obtain an antigen solution suitable for immunizing a mammal.

The resultant pili component antigen solution (1.0 ml) was injected under the skin on the back of a rabbit having a body weight of about 3 kg in conventional manner. A similar immunization was effected 3 times in total with an interval of 4 weeks. 4 weeks after the final immunization, the animal was sacrificed by cardiac puncture and the blood was collected from the animal, to which ammonium sulfate was added at a saturation of 33%. The blood was agitated to dissolve ammonium sulfate and then allowed to stand at 4°C for 48 hours. The precipitate was collected by centrifugation (8000 r.p.m./20 min.) and put into a cellophane tube for dialysis which was effected at 4°C against purified water to remove ammonium sulfate completely. The residual solution was collected, concentrated and freeze-dried to obtain an antibody-containing solution (about 85 ml).

Example 4

Preparation of Antibodies using PLS antigen II isolated from *S. mutans KH2*

In a similar manner to that described in Example 3, *Streptococcus mutans* KH2 (FERM-P No. 366; serotype *d*) was cultured and treated to obtain an antibody-containing solution (about 85 ml).

Example 5

A baccal was prepared in conventional manner by the use of glucose (1 g), antibody-containing solution (0.05 ml; PLS antigen I antibodies or PLS antigen II antibodies or a combination of such antibodies) and soluble starch (0.05 g).

Example 6

A syrup was prepared in conventional manner by the use of carboxymethyl cellulose (CMC-Na; 0.2 g), 20% fructose solution (20 ml), ethyl paraffin (0.04 g) and antibody-containing solution (0.1 ml; PLS antigen I antibodies or PLS antigen II antibodies or a combination of such antibodies).

Example 7

A dental paste was prepared by mixing uniformly together calcium hydrogen phosphate (fine powder; 60%), glycerol (30%), CMC-Na (10%) and parabens (antiseptic agent; 0.25%) and adding to the mixture antibodies (PLS antigen I or PLS antigen II antibodies or a combination of such antibodies), such that the antibody titre was 2 units (as hereinbefore defined) per 50 ml of the product.

Example 8

A non-cariongenic drink containing lactic acid-producing bacilli was prepared in the following manner. Skimmed milk (2000 ml) containing 10% of solids was sterilized at 110°C for 15 minutes, to which was then added *Lactobacillus casei* for culturing at 35-37°C for 36 hours. To the culture broth was added 10% fructose solution in order to adjust the concentration of *L. casei* to about 10^8 living cells/ml. Antibodies raised against PLS antigens I and II were added to this solution and the titres of PLS antigen I antibodies and PLS antigen II antibodies were both adjusted to 4 units (as hereinbefore defined) per 50 ml of the resultant product.

In the following experiments, compositions containing equal titres of antibodies raised against PLS antigen I and PLS antigen II, prepared by the methods of the above-mentioned examples, were used to investigate the inhibition of adherence of cariogenic *S. mutans*. As test animals, hamsters (male and female; each group consisting of 5 animals) were used unless otherwise specified.

Experiment 1:

Among hamsters (21 days after birth) used as test animals, the first and second groups were test groups and the third and fourth groups were used for control purpose. *Streptococcus mutans* Mutant Strain K-Dp (FERM-BP No. 317) and *Streptococcus mutans* KH2 FERM-P No. 366) were separately cultured for 24 hours at a pH of 7.5-7.8 by using Tryptocase Soy Broth (commercial product of BBL., U.S.A.). Culture broth containing about 10^8 living cells of K-Dp strain per ml was orally administered to each animal of the first and third groups at a dose of 0.1 ml once daily for 5 days. Similarly, KH_2 strain was administered to each animal of the third and fourth groups. The adherence of the strains of *S. mutans* to the teeth of all the animals was confirmed. Then, a dental paste prepared by the method of Example 7 (0.1 g per animal; containing equal titres of PLS antigen I and PLS antigen II antibodies) was applied heavily to the molar surfaces of each animal of the first and second groups by means of a small brush. The application was effected once daily for 14 days.

During the test period of 60 days, all animals were bred *ad libitum* with a cariogenic diet (Diet 2000, commercial product of Funabashi Nojo, Japan) and deionized water. Samples were collected after various periods from the molar surfaces of each animal. Each sample was cultured for 72 hours using TYC agar plate medium (15 ml, pH 7.4; Stoppelaar et al) and Mitis-salivarius agar plate medium (15 ml, pH 7.4; commercially available from Difco., U.S.A.) in order to investigate the adherence of *S. mutans*. It was observed that the concentration of *S. mutans* in the oral cavity of each animal of the first and third groups gradually decreased by administration of the antibodies. Strains of *S. mutans* disappeared from the

proportion of the animals after about 2-4 weeks and from the remaining animals after about 4-7 weeks. No decrease of the concentration of *S. mutans* in the oral flora of any animal of the control groups was found. After completion of the test, each animal was anesthetized by injection of pentabarbital and the maxilla was collected from each animal. The soft part of the maxilla was removed by treating at 120-125°C for 1-2 minutes in an autoclave. The maxilla was washed with water and dried to prepare a sample of the teeth. All molars of each animal were examined to determine the ratio of infection (adherence) with *S. mutans* and the ratio of induction of dental caries with reference to the number of carious teeth. The results are indicated in the following Table 1.

TABLE 1

Group	*Antibodies used*	*Serotype of S. mutans*
1	I and II	c
2	I and II	d
3	untreated	c
4	untreated	d

Group	*A*	*B*	*C*
1	77.2	20	7.5
2	74.2	0	0
3	77.4	90	65
4	74.0	70	34.2

Notes:

A... increase of body weight (average %; body weight before the beginning of administration = 100%).

B... infection ratio (all molars = 100%)

C... carious ratio (all molars = 100%)

Experiment 2:

The change in the concentration of the strains of *S. mutans* in the oral cavity of humans resulting from use of the dental paste described in Example 7 (containing equal titres of PLS antigen I and PLS antigen II antibodies) was investigated. The test panel consisted of 10 adults (5 men and 5 women). From the oral cavity of each adult, dental plaque was collected three times, and the samples were cultured in a similar manner to that described in Experiment 1. Moreover, the formation of dental plaque and cysts in the oral cavity was investigated as set out in "The guide line of the procedure of smearing fluoride" issued by The Japanese Ministry of Welfare and Health. As a result, the existence of strains of *S. mutans* in all the adults was comfirmed. Every day after breakfast and dinner, the dental paste was applied by all members of the test group to their teeth. The method of application was not specified. At least once per week, dental plaque was collected from the oral cavity of each member and cultured in a similar manner to that described above in order to investigate the change in the concentration of the strains of *Streptococcus mutans* in the oral flora. All or almost all strains of *S. mutans* disappeared from 3 members after about 2-3 weeks and from the remaining members after about 4-7 weeks. The OHI values of all members, which were determined by using erythrosine, decreased significantly over the test period.

Experiment 3:

A baccal prepared by the method of Example 5 and containing both PLS antigen I and PLS antigen II antibodies was orally administered to each member of a test group consisting of 20 women (adults) and the change in the concentration of *Streptococcus mutans* in their oral cavities investigated. 19 women were the hosts of *S. mutans* of serotype *c* and one woman only was the host of *S. mutans* of serotype *d*.

Every day at bed-time, a baccal was administered orally to each member of the test group. The baccal was kept in the oral cavity for as long as possible. About 2 weeks after the beginning of administration, no *S. mutans* could be isolated from 5 members of the test group. The concentration of *S. mutans* in the oral flora of the remaining members decreased gradually so that about 4 weeks after the beginning of administration, the concentration of *S. mutans* in their oral cavities was very low. The OHI values of all members decreased greatly over the test period.

It was noted that the inhibiting effect resulting from the administration of baccals was at least equal or superior to the effect of dental paste, as the stay time of a baccal in the oral cavity is longer than th stay time of usual dentifrices.

CLAIMS

1. A process for the preparation of antibodies for inhibiting human dental caries induced by *Streptococcus mutans*, which comprises immunizing a mammal with at least one antigen selected from two antigens, a first of which is isolated from the pili-like structures on the cell surface of strains of *S. mutans* of serotypes *c, e, f* and *g* and a second of which is isolated from the pili-like structures on the c ll surface of strains of *S. mutans* of serotype *d*, and recovering the resultant antibodies from said mammal.

2. A process as claimed in claim 1, wherein said two antigens are acidic glyco-proteins, comprising about 15-25% protein and about 75-85% carbohydrates and having a molecular weight of about 50,000-90,000 and a specific gravity of about 1.3-1.4.

3. A process as claimed in claim 1 or claim 2, wherein a mutant strain of *S. mutans* is employed.

4. A process as claimed in claim 3, wherein said mutant strain is *Streptococcus mutans* Mutant Strain K-Dp (FERM-BP No. 317).

5. A process as claimed in claim 3, wherein said mutant strain is *Streptococcus mutans* Strain KH2 (FERM-BP No. 366).

6. A process as claimed in any of the claims 1 to 5, wherein isolation of the desired antigen or antigens is effected by extraction with a hypertonic saline or buffer solution followed by density gradient centrifugation.

7. A process as claimed in claim 6, wherein said isolation is effected at a temperature of not higher than 10°C.

8. A non-cariogenic composition, for inhibiting human dental caries induced by *Streptococcus mutans*, wherein the active ingredient comprises antibodies corresponding to at least one antigen as defined in claim 1 in association with a pharmaceutically acceptable carrier or excipient.

9. A composition as claimed in claim 8 in a form suitable for oral administration.

10. A composition as claimed in claim 9, in the form of chewing gums, candies, icecreams, syrups, juices and drinks containing lactic acid-producing living bacilli.

11. A composition as claimed in claim 9 in the form of dentrifices, gargles and pastes.

12. A composition as claimed in claim 9 in a form selected from baccals and troches.

13. The mutant strain of *Streptococcus mutans* designated *Streptococcus mutans* Mutant Strain K-DP (FERM-BP No. 317) and mutants thereof which may be employed for the preparation of antibodies as defined in claim 1.

14. The mutant strain of *Streptococcus mutans* designated *Streptococcus mutans* KH2 (FERM-BP No. 366) and mutants thereof which may be employed for the preparation of antibodies as defined in claim 1.

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